exhibiting greater oncotoxicity and less general toxicity than those now available still remains. Our interest in replacing the methyl group of HN2 with physiologically active carrier groups led to the synthesis of 5-bis-(2-chloroethyl)-aminouracil, which has been found to be a particularly active carcinostatic and carcinolytic agent in animals.

5-Bis-(2-chloroethyl)-aminouracil (I) was prepared by treating 5-aminouracil with ethylene oxide in aqueous acetic acid to give 5-bis-(2-hydroxyethyl)-aminouracil (II), which was then chlorinated with thionyl chloride in diethylene glycol dimethyl ether in the presence of traces of water and ethanol.

I melts at 206° (dec.); $\lambda_{max.}$ (0.01 N H_2SO_4 in 95% ethanol) 257 mμ a_M 5,675; found C, 48.54; H, 4.44; N, 16.67; Cl, 27.81. II melts at 166–168°; $\lambda_{max.}$ (0.01 N H_2SO_4 in 95% ethanol) 258 mμ—a_M 5,600; 204 mμ—a_M 8,250; found C, 44.50; H, 6.55; N, 19.2.

I has been shown to have an LD_{50} (acute) in rats and mice of about 3.7 mg. per kg. intraperitoneally and about 7.5 mg. per kg. orally. It has minimal hepatotoxicity and shows no automatic or cardiovascular activity in anesthetized dogs. It is not a uracil antagonist for *E. coli* Bu^- which requires this metabolite.

When 5-bis-(2-chloroethyl)-aminouracil is given at the maximum tolerated dose every four to seven days to rats bearing established tumors it is highly effective in causing regression of the tumors without the manifestation of permanent toxicity. The preferred method of dosing is to give the drug orally at 2 mg. per kg. every four days or at 4 mg. per kg. initially and then after seven days at 2 mg. per kg. every fourth day as required. Using these methods the drug is active as a carcinolytic agent against the Walker 256 carcinoma, Jensen sarcoma,

TABLE I

	Days after tumor implantation	Size of tumors mm.	Regressions
Tumor control	13	19	..
	20	39	..
	45	79	..
5-Bis-(2′-chloroethyl)-aminouracil	13	22	..
	20	17	3
	45	1	7
	50	0	8

Walker 256 carcinoma implanted in male rats. I given in 10% ethanol–90% saline: 4 mg./kg. on 13th day, 2 mg./kg. on 18th and 22nd days. 5/9 control animals dead on 45th day, remainder moribund. 8/9 treated animals alive and free of tumors on 50th day.

and Murphy–Sturm lymphosarcoma when the tumors in each case are 10–20 mm. in diameter and have been growing seven to fourteen days. Table I indicates the type of response found with the Walker tumor and is illustrative of that uniformly obtained in the case of other tumors.

The drug shows a linear dose-response curve with respect to inhibition of early transplants when given daily, but under this method of administration the general toxicity is cumulative and the agent is therefore ineffective in causing regression of established tumors. I has also been shown to cause marked or complete inhibition of a wide spectrum of tumors including Sarcoma 180, Cloudman S91 melanoma, Carcinoma 755, and Leukemia L1210 in mice.

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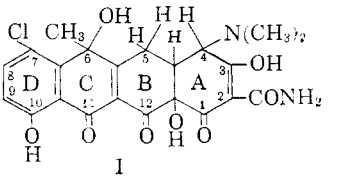
RECEIVED NOVEMBER 7, 1958

THE BIOLOGICAL REDUCTION OF 7-CHLORO-5a(11a)-DEHYDROTETRACYCLINE TO 7-CHLOROTETRACYCLINE BY STREPTOMYCES AUREOFACIENS

Sir:

We have reported recently[1] the characteristics and structure of 7-chloro-5a(11a)-dehydrotetracycline (I), a new tetracycline-like material accumulated by a blocked mutant of *Streptomyces aureofaciens* Duggar, coded as mutant S-1308 and descended from the original 7-chlorotetracycline-producing[2] A-377 soil isolate of Duggar. We also have demonstrated[1] that catalytic hydrogenation of I yields a mixture of approximately equimolar quantities of tetracycline[2] and its epimer, 5a-*epi*-tetracycline.



I

We now wish to describe the biological reduction of I to 7-chlorotetracycline by two *S. aureofaciens* mutants. They are mutant BC-41, a producer of 7-chlorotetracycline, and mutant V-138, a producer of 7-chloro-6-demethyltetracycline[3]; both are descended from the original A-377 soil isolate of Duggar. Addition of I (500 μg./ml.) to 48-hour old fermentation systems, followed by 72 hours of additional fermentation, resulted in reductions by BC-41 and V-138 of 40% and 20% of added I, respectively, the reduction product in both cases being 7-chlorotetracycline. The extents of reduction were estimated by measuring the 7-chlorotetracycline produced; these measurements were carried out by fluorometric assay,[4] by quantitative paper strip chromatography, and by radiochemical methods utilizing 7-chloro^{36}-5a(11a)-dehydrotetracycline. In the radiochemical experiments, no radiochloride ion was observed at the end of the fermentation, the only labeled substances being 7-chlorotetracycline product and unchanged starting material. This observation, taken with the fact that the fermentation contained a large excess of unlabeled chloride, shows that degradation of I to chloride ion, followed by resynthesis to labeled 7-chlorotetracycline, was not occurring. In a similar experiment, BC-41 was grown in the pres-

(1) J. R. D. McCormick, P. A. Miller, J. A. Growich, J. Reichenthal, N. O. Sjolander and A. P. Doerschuk, THIS JOURNAL, **80**, 5572 (1958).

(2) The trademarks of the American Cyanamid Company for 7-chlorotetracycline and tetracycline are Aureomycin and Achromycin, respectively.

(3) J. R. D. McCormick, N. O. Sjolander, U. Hirsch, E. R. Jensen and A. P. Doerschuk, THIS JOURNAL, **79**, 4561 (1957).

(4) D. H. Feldman, H. S. Kelsey, and J. C. Cavagnol, *Anal. Chem.*, **29**, 1697 (1957).

ence of a chlorination inhibitor[5] to direct the fermentation from the production of 7-chlorotetracycline to the production of tetracycline. Again, the reduction product from added I was observed to be the chlorinated product, 7-chlorotetracycline.

Complete reduction of I never was observed despite the fact that, during the reduction periods, the organisms synthesized endogenous tetracyclines to the extent of 5 to 30 times the quantity of I reduced. This was not due to the presence of a large pool of I, since no appreciable quantities of I have been observed during fermentations of BC-41 and V-138. These organisms reduced I only when it was present during that phase of the fermentation in which endogenous tetracyclines were being actively produced. The biological reduction yielded only 7-chlorotetracycline; in contrast, catalytic hydrogenation, under previously reported conditions,[1] yielded both of the epimers at C.5a and removed chlorine.

It is suggested that 7-chloro-5a-(11a)-dehydrotetracycline is a precursor of 7-chlorotetracycline and that, possibly, the last step in 7-chlorotetracycline biosynthesis is the reduction of the 5a(11a) double bond in 7-chloro-5a(11a)-dehydrotetracycline.

(5) Y. Sekizawa, *The Journal of Biochemistry (Japan)*, **42**, No. 2, 217 (1955).

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RECEIVED NOVEMBER 6, 1958

A NEW EPOXY ALDEHYDE: SYNTHESIS OF GLYCIDALDEHYDE FROM ACROLEIN AND HYDROGEN PEROXIDE

Sir:

Although the epoxidation of α,β-unsaturated ketones by alkaline hydrogen peroxide is a well known procedure,[1] the corresponding reaction with *simple* α,β-unsaturated aldehydes has not been described.[2]

We wish to report the synthesis of glycidaldehyde (I) from acrolein and hydrogen peroxide. Equimolar amounts of these materials were combined at room temperature and added dropwise with stirring over 1 hour at 25–30° to an aqueous solution held at *p*H 8–8.5 by the continuous addition of *N* sodium hydroxide. After an additional 0.5 hour, titration for oxirane oxygen indicated an 82% yield of I. Anhydrous glycidaldehyde, a

$$H_2C{=}CH{-}CHO + H_2O_2 \longrightarrow \underset{\text{I}}{H_2C\overset{O}{\diagup\diagdown}CH{-}CHO}$$

compound heretofore not described in the chemical literature, was secured in 33% recovery by saturation of the reaction mixture with ammonium sulfate, extraction with warm cyclohexanone, and fractional distillation. It is a colorless stable liquid with a pungent odor having b.p. 112–113° (760 mm.) and 57–58° (100 mm.), $n^{20}D$ 1.4185, sp. gr.$^{20}_4$ 1.126. (Calcd. for $C_3H_4O_2$: C, 50.0; H, 5.6; oxirane oxygen, 22.2; carbonyl value, 1.39 equiv./100 g. Found: C, 50.1; H, 5.7; oxirane oxygen, 21.8; carbonyl value, 1.39 equiv./100 g.). The 2,4-dinitrophenylhydrazone derivative had m.p. 96–98° followed by resolidification and m.p. unsharp *ca.* 150° (Calcd. for $C_9H_8N_4O_5$: C, 42.9; H, 3.2; N, 22.2. Found: C, 42.9; H, 3.2; N, 22.1).

A 10% aqueous solution of glycidaldehyde underwent hydrolysis at a rate of about 0.4% per day when stored at 5°. The hydrolysis product, glyceraldehyde, had m.p. and mixed m.p. 136–138°.

Range finding acute toxicity studies place glycidaldehyde in a moderately toxic class by oral, vapor, and percutaneous routes.

Detailed investigations of both the synthesis and chemical reactions of glycidaldehyde have been carried out and will be reported at a later date.

(1) E. Weitz and A. Scheffer, *Ber.*, **54**, 2327 (1921); they obtained only acidic products from crotonaldehyde and cinnamaldehyde.

(2) 2,3-Diphenylacrolein has recently been epoxidized; see Absts. of the 134th A.C.S. Meeting, Sept. 7–12, 1958, p. 28-P.

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RECEIVED SEPTEMBER 2, 1958

CHEMISTRY OF THE NEOMYCINS. IV. ISOLATION OF NEOSAMINES B AND C, STEREOCHEMISTRY OF NEOBIOSAMINE C

Sir:

It has been shown that neobiosamine C,[1] from the antibiotic neomycin C, is a disaccharide composed of D-ribose[2] and a 2,6-diaminoaldohexose (neosamine C).[3] Neosamine C and the corresponding neosamine B (from neomycin B *via* neobiosamine B)[1] have now been isolated, and the most probable stereochemistry of neobiosamine C has been shown to be that represented by formula I.[3a]

Hydrolysis of methyl neobiosaminide C[1] (III)[3a] for 90 min. in refluxing 6*N* hydrochloric acid gave neosamine C dihydrochloride, $[\alpha]^{23}D$ +67° (*c* 0.87, water). [Found: C, 28.64; H, 6.40; N, 10.75.] The hygroscopic hydrochloride, which gave positive reactions with ninhydrin and aniline acid phthalate,[4] sintered at 140° and darkened, but did not melt below 230°.[5]

Periodate oxidation of N,N′-dibenzoylneosaminol C (IV)[3a] gave N-benzoyl-L-serinaldehyde (negative rotation—*cf.* periodate oxidation of N-benzoyl-D-glucosaminol,[6] identified by papergrams after conversion to serine)[3] from C-1, C-2 and C-3 of neosamine C, while periodate oxidation of methyl N,N′-dibenzoylneobiosaminide C, (II), then bro-

(1) K. L. Rinehart, Jr., P. W. K. Woo, A. D. Argoudelis and A. M. Giesbrecht, THIS JOURNAL, **79**, 4567 (1957).

(2) K. L. Rinehart, Jr., P. W. K. Woo and A. D. Argoudelis, *ibid.*, **79**, 4568 (1957).

(3) K. L. Rinehart, Jr., and P. W. K. Woo, *ibid.*, **80**, 6463 (1958).

(3a) The compound numbers employed refer to formulas found in Neomycins III.[3]

(4) S. M. Partridge, *Nature*, **164**, 443 (1949).

(5) It has been reported [J. D. Dutcher, N. Hosansky, M. N. Donin and O. Wintersteiner, THIS JOURNAL, **73**, 1384 (1951)] that vigorous hydrochloric acid hydrolysis of methyl neobiosaminide C yielded the dihydrochloride of a reducing diamine, $[\alpha]^{32}D$ +69° (*c* 0.4 water) s. 155–175°, m.p. 182–185° dec. Analytical values of this material suggested the formula $C_8H_{14}N_2O_3{\cdot}2HCl$, that of a desoxydiaminohexose [however, *cf.* Ref. (1)].

(6) W. E. M. Lands, Ph.D. Thesis, University of Illinois, 1954.